REMARKS

Claims 1-18 and 20-26 were rejected under 35 USC 103(a) as being unpatentable over Cleuziat et al. (USPN 5,849,547) and in view of Walker et al. (EP 0 500 224).

First, neither Cleuziat et al and Walker disclose or make obvious a method of DNA amplification that uses both a pair of long primers each with a digestion resistant region in combination with a pair of short primers that a specifically selected to include the a sequence homology with the digestible regions of the long primers. Secondly, the two references, Cleuziat et al, and Walker should not be combined because to do so would, in fact, make the method described by Cleuziat et al in operable.

The present invention uses long first and second primers (A-B and C-D, respectively) that hybridize to the 3'binding regions of the separated strands of DNA. Each of the first and second primers includes a digestion resistant region remote from its 5' end. Referring to Fig. 3 steps a and b of the present application, after the first and second primers hybridize to the individual strands extension of the primers by the polymerase provides two partial double stranded DNA products, each with a primer that includes a digestion resistant region. The 5' double strand specific exonuclease then removes those regions on each of the four strands until the digestion resistant region is encountered (step c). This exposes the regions of the strands with complimentary sequence to which two additional long primers can hybridize (step d). Extension of the newly hybridized long primers provides two double stranded DNA products in which each strand includes a digestion resistant region (step e). Now, digestion by the 5' specific exonuclease reveals only a short binding site on each strand that is specific for the short primers, i.e., primers A and C (steps f and g). Primers A and C have been specifically selected to have a degree of homology with the digestible regions of the long first and second primers. The last sequence of step is repeated in steps 3 (j and k).

Cleuziat et al. as noted does not disclose or teach an amplification method that uses either a primer with a digestion resistant region or the use of an enzyme exhibiting 5' double strand specific exonuclease activity. (Office Action, page 3, lines 16-18.) Additionally, Cleuziat et al does not disclose or teach the use of a short primer that has a degree of homology with the digestible regions of the [long] primers having the digestion resistant regions. Cleuziat et al does disclose several "short" primers, C-F. However, none of these "short" primers as described as having a degree of homology with the digestible regions of the long primer.

This deficiency is not rectified by Walker. Walker discloses a method using modified nucleotides that inhibit digestion of the strand containing the substituted deoxynucleosidetriphosphates (dNTP's). Walker merely discloses that the "modified nucleotides can alternatively be incorporated into the primer during chemical synthesis. In this case, the modified deoxynucleosidetriphosphates are not necessary." (Walker, col. 8, lines 42-45.). However, Walker does not disclose or teach how to use the primers that include the modified dNTP's. For the purposes of the following discussion we will refer to these primers that include modified nNTP's as A' and B'. Referring now to Walker, Fig. 2, steps 1-5, if primers A' and B' that include the modified dNTP's that resist digestion are hybridized to the single strands--instead of primers A and B in combination with the modified dNTP's in the solution as illustrated in Fig.3--then digestion by the 5'-3' exonuclease would expose only a portion of the region to which the modified primers A' and B'. Walker provides no suggestion or teaching as to what would bind to these exposed regions. Presumable, the dNTP's in solution would bind there or possible a portion of the A' and B' primers. However if new primers A' and B' were to bind to the exposed regions, only the 5' end of the primers could bind because the original A' and B' primers would still be bound at their 3' ends. In other words, only complementary strand to the 5' digestible portion of the primers would be exposed and available for and binding. Therefore only a portion of a new A' (or B') primer could bind there. The effect is that even should a new A' primer bind to the exposed region, the 3' end of the newly bound primer would not bind. Since the 3' end contains the polymerase promoter, this newly bound primer could not be extended to generate a copy of the target sequence. Further, the extended strand incorporating the partly digested A' primer might not be displaced from the target DNA fragment.

Consequently Walker only mentions that modified nucleotides can be incorporated into the primers. Walker does not disclose nor teach how these modified primers could be used to amplify a target DNA strand. Further and possible of greater importance, Walker does not disclose or teach use of "short primers" specifically selected to have a sequence homology with the digestible regions of modified primers.

Cleuziat et al does not disclose or teach use of either the long primers modified to have 5' double stranded exonuclease digestion resistant regions or the short primers that have a sequence homology with the digestible region of the long primers.

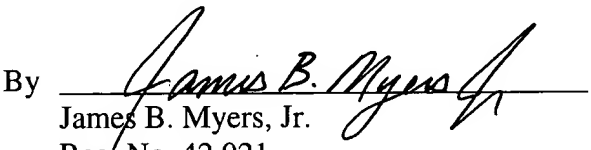
Additionally, Walker cannot be combined with Cleuziat et al. because to do so would change the basic operation of the Cleuziat method. The DNA amplification method described by Cleuziat et al. beginning at col. 15, lines 12- 45 was specifically designed to be used without exonuclease activity. Instead Cleuziat uses a number of different primers, A-F, each which includes a polymerase promoter sequence. To this end the Cleuziat et al method uses a primers A and B to bind to the single stranded DNA fragment. Primers A and B hybridize to the single strands and are extended along the length of the target strands. The secondary primers "G" and "H" hybridize upstream of the respective primers A and B. (See also Cleuziate et al., col. 11, lines 46-50 for the definition of "upstream" and "downstream".) It is important to recognize that the primers G and H do not hybridize at the same regions as primers A and B. Consequently if the modified primers of Walker and the exonuclease activity were used in Cleuziat method, then the modified A' primer would hybridize to the single strand and be extended by the polymerase. Digestion by the 5'-double strand exonuclease would remove the digestible portion of the modified A' primer, expose the corresponding region of the complimentary DNA strand, and digest the downstream portion of the same complimentary DNA strand. The "G" primer would hybridize on the complementary strand and be located to the 5' side of the undigested A' primer and, therefore, not to hybridize region on the complimentary strand exposed as a result of the exonuclease activity. It is uncertain what would bind to this exposed site on the complementary strand. Further, the exonuclease would digest the bulk of complementary strand of the DNA, i.e. the 5' end of the complementary DNA strand. This would inhibit extension of the G primer because there is no template upon which to extend the G primer. This would ultimately inhibit displacement of the strand obtained from partly digested (and extended) A primer. If however, this strand were displaced from the target sequence, the now freed single strand DNA fragment incorporating the partly digested A' primer would not function in the remaining amplification sequence the same as the DNA fragment that incorporates the full A primer, i.e., fragment II in Fig. 3 of Cleuziat et al.

Neither Walker nor Cleuziat et al discloses or teaches the use of a (long) primer with an exonuclease digestible resistant region, a second primer with a sequence homology to the digestion region of the first (long) primer, and an exonuclease to amplify a target DNA sequence. Consequently, withdrawal of the rejections is requested.

Conclusion

In view of the foregoing remarks, the Applicants respectfully submit that the cited references, either singly, or in combination, do not disclose, or make obvious the claimed invention. Accordingly, reconsideration leading to withdraw of all the rejections under 35 U.S.C. § 103(a) and passage of this application containing claims 1-18 and 20-26 is requested. Additionally, the Examiner is invited to telephone the undersigned attorney if there are any questions about this submission or other matters, which may be addressed in that fashion.

Respectfully submitted,

By 

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